Potential Protective Influence of Combined Vitamin C Against the Rhodamine B Dyes on Fertility in Male Rats

Amal Z. Nasef , Emad M. El-Kholie and Hend S. Mandour
Nutrition and Food Science, Faculty of Home Economics, Menoufia University, Egypt

المجلة العلمية المحكمة لدراسات وبحوث التربية النوعية

المجلد العاشر – العدد الرابع – الجزء الثاني – مسلسل العدد (٢٧) – أكتوبر ٢٠٢٤م

رقم الإيداع بدار الكتب ٢٤٢٧٤ لسنة ٢٠١٦

ISSN-Print: 2356-8690 ISSN-Online: 2974-4423

موقع المجلة عبر بنك المعرفة المصري https://jsezu.journals.ekb.eg

البريد الإلكتروني للمجلة E-mail JSROSE@foe.zu.edu.eg

Potential Protective Influence of Combined Vitamin C Against the Rhodamine B Dyes on Fertility in Male Rats

Amal Z. Nasef , Emad M. El-Kholie and Hend S. Mandour
Nutrition and Food Science, Faculty of Home Economics, Menoufia University, Egypt

Abstract

Rhodamine B is one of the industrial food dyes that give cotton candy its bright red color. Antioxidants such as Vitamin C delay or inhibit cell damage primarily through the property of removing free radicals, which alleviate any form of oxidative stress. The aim of the study is to evaluate the effect of daily consumption of rhodamine B with or without vitamin C on some testicular biomarkers, including reproductive organ weight, reproductive hormone levels, and testicular histological examination. Forty-Two adult male rats type albino weighting (150±10g) was divided into 7groups, each group consists of six rats as follows: Group 1: fed on basal diet and used as control (- ve) group. Groups (2,3, and4): fed on basal diet and administrated only rhodamine B at dose 10, 20, and 30mg., respectively. Groups (5,6, and7): fed on basal diet, and administrated rhodamine B at dose 10, 20, and 30mg/200g b.w. and kiwi juice at 42g/200g BW as a source of vitamin C, respectively. At the end of experiment(36 day), Blood samples were taken after the experiment to assess serum testosterone (TH), luteinizing hormones (LH), follicle stimulating hormone (FSH), serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), oxidative enzyme activity such as superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), lipid profile, blood parameters ,histopathological and biological investigation were also measured. Results indicated that the addition of vit. C improved enzymes CAT and SOD and decreased MDA, also, reduced the harmful effect of rhodamine B on liver enzymes. The intake of vit. C also improved hyperlipidemia, blood parameters, as well as a significant increase in weight, feed intake and food efficiency and improved sex hormones of rats received rhodamine dye and vit. C, which led to a decrease in the negative effect of dye levels.

Keywords: Fertility- Rhodamine -sex hormones – vitamin - testosterone

التأثير الوقائي المحتمل لفيتامين سي المركب ضد تأثير صبغة الرودامين ب على الخصوبة في ذكور الفئران

الملخص

يعد رودامين ب أحد الأصباغ الغذائية الصناعية و التي تعطي الحلوي لونها الأحمر الزاهي. وتعمل مضادات الأكسدة مثل فيتامين سي على تأخير أو تثبيط تلف الخلايا في المقام

الأول بواسطة خاصية إزالة الجذور الحرة، مما يخفف من أي شكل من أشكال الإجهاد التأكسدي. الهدف من الدراسة هو تقييم تأثير الاستهلاك اليومي للرودامين ب مع أو بدون فيتامين سي على بعض المؤشرات الحيوية للخصية، بما في ذلك وزن الأعضاء التناسلية، ومستويات الهرمونات الجنسية، والفحص النسيجي للخصية. تم تقسيم ٤٢ من الفئران الذكور البالغة من نوع ألبينو بوزن (١٥٠ ± ١٠ جم) إلى ٧ مجموعات، تتكون كل مجموعة من ستة فئران على النحو التالي: المجموعة ١: تم تغذيتها على النظام الغذائي الأساسي واستخدمت كمجموعة ضابطه سالبه. المجموعات (٢،٣، و٤): تم تغذيتها على النظام الغذائي الأساسي وإعطائها رودامين ب فقط بجرعات ١٠، ٢٠، ٣٠ مجم على التوالي. المجموعات (٥،٦، و٧): تم تغذيتها على النظام الغذائي الأساسي وإعطائها رودامين ب بجرعات ١٠، ٢٠، ٣٠ مجم من وزن الجسم وعصير الكيوي بجرعة ٤٢ جم / ٢٠٠ جم من وزن الجسم كمصدر لفيتامين سي على التوالي. في نهاية التجربة (٣٦ يوم)، تم أخذ عينات دم بعد التجربة لقياس مستويات هرمون التستوستيرون، والهرمون الملوتن ، والهرمون المنبه للجريب ، وأسبارتات أمينوترانسفيراز المصل ، وألانين أمينوترانسفيراز، ونشاط الإنزيمات المؤكسدة مثل سوبر أكسيد ديسميوتاز ، والكاتالاز ، والمالونديالدهيد ، ودهون الدم ، وصوره الدم الكامله، وكذلك الفحص النسيجي للخصيه والتقييم والبيولوجي. أشارت النتائج إلى أن إضافة فيتامين سي أدى أيضًا إلى تحسين هذه الإنزيمات CAT وSOD وخفض قيم MDA، وتقليل التأثير الضار لرودامين ب على إنزيمات الكبد. كما أدى تناول فيتامين سي إلى تحسين صورة دهون الدم وصوره الدم الكاملة، فضلاً عن زيادة كبيرة في الوزن وتناول العلف وزيادة كفاءة الغذاء وتحسن الهرمونات الجنسية للفئران التي تلقت صبغة رودامين وفيتامين سي، مما أدى إلى انخفاض التأثير السلبي لمستويات الصبغة.

الكلمات المفتاحية: الخصوبة – الرودامين – الهرمونات الجنسية – الفيتامين – التستوستيرون

Introduction

Rhodamine B is a textile dye that is harmful to people's health. It frequently enters the body when paired with food and causes oxidative stress, which strains cells and tissues. Rhodamine B poisoning can occur from short-term exposure to high amounts, whereas cancer or liver damage can result from long-term ingestion of food containing the compound **(Mahdi *et al.*, 2019).** The use of rhodamine B as a coloring agent is forbidden by PERMENKES RI No.239/Menkes/Per/V/1985. It is a kind of dye that can be harmful to people's health if added to food or drink. Rhodamine B accumulates in the liver due to the liver's inability to metabolize it, which results in liver disease. Rhodamine B's chemical structure includes the carcinogenic element N+ (nitronium), which

promotes the growth of cancer cells and results in cancer and liver tumors. **(Hadriyati, 2021).** Foods that include rhodamine B have vivid, shiny, and more noticeable colors; occasionally, the color may appear uneven or have color clumps in the meal, and when consumed, the taste may taste slightly bitter. Food items that include this ingredient typically don't have a code, label, brand, or other comprehensive identification **(Adriani and Pendidikan, 2019).**

Rhodamine B dye is employed in the production of a variety of items, including ballpoint pens, paints, leather goods, dye lasers, carbon sheets, ink for stamp pads, fireworks, and explosives (Hamdaoui, 2011; Imam and Babamale, 2020). Among these, synthetic dyes represent one of the most hazardous pollutants, and their application is increasing due to their significant demand across multiple industrial sectors, including paper, cosmetics, textiles, leather, and food. Consequently, Varjani et al. (2020) anticipate an increase in wastewater generation from the dye manufacturing industry. Rhodamine B is often found in aquatic environments, which may be dangerous for animal and human health. It results in carcinogenic and mutagenic changes in biological organisms **(Zhao *et al.*, 2013; Vu˘curovi´c *et al.*, 2014 and Goscianska *et al.*, 2015).** It is also a neurotoxic dye in both humans and animals, causing irritation of the eyes, skin, gastrointestinal tract, and respiratory tract (Jyotshana et al., 2022).

Global reports indicate a decline in male fertility **(Sengupta, 2017)** associated with multiple interfered factors related to genetic, environmental and nutritional ones. According to estimates of the World Health Organization (WHO), between 45 and 80 million couples worldwide are infertile **(Rutstein & Shah, 2004 and Agarwal *et al.*, 2015).** The ability to achieve a clinical pregnancy is known as fertility **(Zegers, 2017).** Due to indications of declining semen quality among young in healthy men, the problem of male infertility has received increased attention globally **(Winters and Walsh,2014).** The rates of male infertility have been reported to be in the range from 2.5% to 12%, in Central/Eastern Europe while Africa is having the highest rate. Males can be the only factor in 20% to 30% of cases of infertility, accounting for around 50% of infertility in couples **(Agarwal *et al.*, 2015).** The prevalence rates in other locations, including in North America, and Australia has ranged from 4.5-6%, 9%, and 8-12%, respectively. Seminal quality, which is indicative of fertility, is a key factor in determining male infertility, albeit other factors dependent on the couple's situation also play a role **(Levine *et al.*,2015).** The following guidelines on problems impacting man fertility: obesity, tobacco use, alcohol use, caffeine use, disordered reproductive hormones, stress induced through oxidative

reactive oxygen species, and a shortage of antioxidants **(Davidand Jessy, 2016).**

Male infertility or infertility in humans is caused by exposure to cadmium, rhodamine, smoking, contaminated food, and damage or disturbance of the testis' vascular system **(Nasef and El-Sheikh, 2023)**.
Antioxidants are the molecules that prevent cellular damage caused by oxidation of
other molecules. Oxidation is a chemical reaction that transfers electrons from one
molecule to an oxidizing agent. Oxidation reactions are known to produce free radi-
cals. These free radicals are highly reactive species which contains one or more
unpaired electrons in their outermost shell. Once they are formed, the chain reaction
starts. Antioxidant reacts with these free radicals and terminates this chain reaction
by removing free radical intermediates and inhibits other oxidation reactions by
oxidizing themselves.

Antioxidants are molecules that play a role in safeguarding cells from damage due to the oxidation of other substances. Oxidation refers to a chemical process where electrons are transferred from one molecule to an oxidizing agent. It is well understood that oxidation reactions can generate free radicals. These free radicals are quite reactive entities that possess one or more unpaired electrons in their outermost shell. Once they emerge, a chain reaction may commence. Antioxidants engage with these free radicals and help to halt this chain reaction by eliminating free radical intermediates and preventing further oxidation reactions through the oxidation of themselves (Mamta et al., 2014).

Kiwis are a fruit high in nutrients; a plethora of study conducted in the past ten years on their health benefits has connected regular kiwifruit consumption to improvements in immunological, metabolic, and digestive health as well as improved nutritional status. Fruit consumption has numerous proven health advantages **(Boeing *et al.*, 2012).** In addition to having high vitamin C content, kiwis also contain a variety of other nutrients, such as dietary fiber, potassium, vitamin E, and folate, all of which are nutritionally significant. They also contain a wide range of bioactive components, such as enzymes, phytonutrients, and antioxidants, all of which have a positive impact on metabolism and function. A rising corpus of research from studies of human intervention is highlighting the role that kiwifruit plays in digestive health. Actinidin, a naturally

occurring proteolytic enzyme specific to kiwifruit that breaks down protein and aids in stomach and digestion, is one of several conceivable modes of metabolism that are likely to work in concert **(Kaur *et al.*, 2010).** The most unique nutritional characteristic of kiwifruit is its total ascorbic acid content **(Boland *et al.*, 2013).**

Materials and Methods

Materials

Rhodamine B dye

It was purchased from a chemical company on Al-Gish Street, Ataba, Cairo, in December 2023.

Experiment animals

Forty-two adult normal male albino rats of the Sprague Dawley strain, weighing 150±10 g each, were purchased from the Vaccine and Immunity Organization, Ministry of Health, Helwan Farm, Cairo, Egypt.

Vitamin C

The fresh fruit of kiwi (*Actinidia deliciosa*) was obtained from local market, Shebin El-Kom City, Egypt, was used as a source of vitamin C.

The kits

Chemical kits had been obtained from Al-Gomhoria Company for Trading Drug, Chemicals, and Medical Instruments, Cairo, Egypt.

Methods

Preparation of Rhodamine B:

Rhodamine B was dissolved in double-distilled water and administered orally using a probe. The treatment group's administration duration of rhodamine B is based on prior research concerning sub-chronic toxicity tests, which involved administering rhodamine B for a duration of 36 days (Siswati and Slamet, 2000).

Preparation of kiwi as a source of Vitamin C:

Vitamin C was administrated daily and orally to rats at dose of 200 mg/kg BW **(Aly *et al.*, 2010).** Vitamin C was estimated based on food composition tables for Egypt as follows: 100 grams kiwi containing (95 mg of vitamin C) **(A.R.E. National Nutrition Institute, 2006).** Kiwi fruits are squeezed using an electric blender (Broun blender, model FX3030, Germany) and administered orally using probe all day long.

Diets

Animals were fed a standardized diet. Experimental procedures were in accordance with the guidelines outlined in National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Ethical approval

Biological experiments conducted for this research received ethical approval from the Scientific Research Ethics Committee (Animal Care

and Use) at Faculty of Home Economics, Menoufia Uni., Shebin El-Kom, Egypt (Approval no. 17- SREC- 08-2023).

Experimental design

Forty two adult male rats type albino weight ranges between (150g ±10g during the experiment period (36 days). Rats will feed on a basal diet for 7 consecutive days to adapt, and rats will be separated into 7 groups, each consisting of six rats, as follows:

Rats were randomly divided into the following 7 groups: Group 1: the control (-ve) group (normal rats. Groups (2, 3, and4). The rhodamine B administration group at dose 10, 20, and 30mg/200g b.w., respectively. Groups (5, 6, and7). They are also given Rhodamine B at dose 10, 20, and 30 mg/200 g b.w., respectively with vitamin C at dose (200mg/kg b.w.) all day long.

Biological indicators

At the end of the trial period, the rate of change in body weight (BWG), Feed Intake (FI), Feed Efficiency Ratio (FER) and were calculated as stated by Chapman et al. (1959), according to the following formula:

$$\text{BWG} = \text{Final weight} - \text{Initial weight}$$

$$\text{FER} = \frac{\text{Gain in body weight (g/rat)/36}}{\text{Feed consumed (g/rat)/36}} \times 100$$

Blood sample collection

Regarding blood tests, blood samples from each rat's hepatic portal vein were obtained following a 12-hour fast. The blood samples were split into two parts. To calculate the Automated Complete Blood Count (CBC), the 1st part component was drawn into a heparinized tube. The second part was drawn into clean, dry centrifuge tubes, and then centrifuged for 10 minutes at 4000 rpm to extract the serum. According to **Schermer (1967)** the serum was carefully collected into clean cuvette tubes and then frozen until examination.

Preparation of testicular

The right testis was homogenized for antioxidant measurement and hormones, whereas the left testis was preserved in 10% formalin for histological inspection. The testis was maintained at -80°C during the preparation of tissue homogenate for antioxidant parameter determinations. The homogenate was spun at 10,000 rpm for 20 minutes.

Biochemical analysis

Determination of testosterone, LH and FSH hormone

Utilizing **Fahim et al. (1982)** method, the calorimetric measurement was done to ascertain the concentrations of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). The technique

delineated by **Predelles et al. (1985)** was implemented to quantitatively identify testosterone hormone via calorimetric analysis.

Determination of oxidative enzymes (CAT, SOD and MAD)

Supernatant was utilized to measure malondialdehyde (MDA) as a marker for lipid peroxidation following the methodology established by Satoh (1978) and to evaluate the antioxidant enzymes catalase (CAT) (Aebi, 1984) and superoxide dismutase (SOD) in accordance with the way outlined by Paoletti and Mocali (1990).

Determination of serum liver enzymes

Liver enzymes are measured using the techniques described in **(Huang et al., 2006; Hafkenscheid, 1979 and Moss, 1982)**, respectively, the serum concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST).

Determination of HB, WBCs and RBCs:

Following the methods of **Dacie and Lewis (2006)**, **Koda-Kimble et al., (2001)**, **and Lubsandorzhiev (2006)**, respectively, hemoglobin, WBCs, and RBCs were measured.

Determination of PLT:

Daly (2011) methods were used, respectively, to determine serum platelet count.

Lipid Profile

Triglyceride enzymatic calorimetric analysis was performed in accordance with **Fassati and Prencipe (1982).** Total cholesterol (TC) was determined according to **Allain *et al*. (1974).** HDL-c was determined according to **Lopez, (1977).** LDL-c and VLDL- c were calculated as stated to **Lee and Nieman (1996).**

Histopathological examination

Rats in various groups had their testicles removed, and the samples were preserved in 10% formalin. They were then given a tap water wash and placed in a bath that contained progressive alcohol dilutions (methyl, ethyl, and 100% ethyl) for the purpose of dehydration. Samples were embedded in liquid paraffin at 56 °C after being cleaned in xylene. Subsequently, 4µm-thick sections were cut, deparaffinized, and dyed with Hematoxylin/Eosin stains for light-microscopy histological analysis **(Bancroft and Gamble, 2008).**

Statistical analysis

Using SPSS software (Version 20; Untitled; SPSS Data Editor), statistical analysis was conducted. The results were articulated as mean ± standard deviation (mean ± SD). An analysis of variance (ANOVA) along with one-way classification was employed for data evaluation. The Duncan test was implemented to assess whether the differences in means were statistically significant at $P<0.05$.

Results and Discussion

The influence of Rhodamine B dye with and without Vitamin C on TH, LH and FSH hormone of male rats

The results revealing the mean values of testosterone (TH), luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH) are presented in Tables (1). A significant increase was observed in the testosterone hormone in control group while significant decrease in, Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH). Their mean values were 5.73 ng-ml, 5.19 IU-ml and 7.33 m IU -ml respectively. However, the high level of RHB produced a significant decrease in the mean value of testosterone (TH) and significant increase in both the other hormones, the mean values were 1.36 ng-ml, 15.29 IU-ml and 24.11 m IU -ml respectively. The differences in mean values between control and dealt with different groups doses of dye (10, 20 and 30 mg) were significant. On the other hand, sexual hormones were improved in rats that received RHB dye and 200mg vitamin C which decreased the negative effect of the levels of dye with significant changes. The improvement percentage of TH at the high dose was about 67% while the reduction percentage of the other hormones were 20 and 13 % respectively as compared the levels of hormones without vitamin C. Also, there is no significant differences between20 mg RhB group and 30 mg RhB with vitamin.

The previous reports consisted of the obtained results, which showed that Rhodamine B poses significant risks to the reproductive system, affecting the hypothalamus, pituitary gland, ovaries, testes, and reproductive tract. Impairment of any of these organs can ultimately result in steroid hormone disruption, potentially leading to ovarian failure. Consequently, the degradation of oocytes disrupts endocrine equilibrium, resulting in diminished levels of estrogen and progesterone, alongside elevated Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH). Cell death can occur through various mechanisms, including necrosis, apoptosis (programmed cell death type-1), and autophagic processes (programmed cell death type-2). In instances of necrosis, a cell exhibits cytoplasmic swelling, disorganized organelle structures, ruptured membranes, and karyolysis of the cell nucleus. Conversely, cells undergoing apoptosis experience distinct changes. The apoptotic pathway is triggered by intracellular regulatory programs, wherein dying cells activate enzymes to degrade nuclear DNA and cytoplasmic proteins. The process of apoptosis is modulated by a variety of proteins, notably the Bcl-2 (B-cell lymphoma-2) protein family **(Baldev et al., 2013 and Wopara et al., 2021).**

Elevated dosage exposure across several generations in rats leads to diminished reproductive capacity and the emergence of tumors in certain specimens after reaching 6 months of age **(Ainslie et al., 2010)**.

Ascorbic acid is a vital water-soluble vitamin that is instrumental in hormone synthesis and collagen formation, while also serving as a protective agent against oxidative stress. It acts as a co-factor in the production of adrenal steroids and the synthesis of catecholamines within the adrenal cortex and medulla, respectively. Ascorbic acid effectively neutralizes free radicals due to its robust antioxidant properties in both intracellular and extracellular aqueous environments. To sustain optimal levels of ascorbic acid, it is imperative that individuals consume vitamin C through their diet, as humans lack the ability to produce it endogenously. The flow of nutrients and dietary consumption of vitamin C have a direct impact on serum ascorbic acid levels, which subsequently affect seminal ascorbic acid concentrations. Numerous studies have identified a correlation between increased dietary vitamin C intake and the enhancement of sperm cellular functionality, reduction of structural defects in sperm cells, decreased sperm DNA fragmentation, and diminished DNA damage **(Carr & Rowe, 2020; Ahn et al., 2021).**

Table (1). The effect of Rhodamine B Dye with or without V.C on TH, LH and FSH hormones of male rat

Parameters / Groups	TH ng-ml	LH IU – mL	FSH m IU -ml
Negative control	5.73±0.17^{a}	5.19±0.05^{f}	7.33±0.59^{f}
10 mg RhB	4.12±0.21^{c}	8.04±0.55^{d}	12.43±1.45^{d}
20 mg RhB	2.45±0.07^{e}	11.13±0.94^{b}	18.88±2.02^{b}
30mg RhB	1.36±0.23^{f}	15.29±0.87^{a}	24.11±0.04^{a}
10mgRhB+200mg vit c/kg BW	4.97±0.11^{b}	7.01±1.01^{e}	9.47±1.34^{e}
20mgRhB+200mg vit c/kg BW	3.63±0.06^{d}	9.39±0.66^{c}	15.27±0.78^{c}
30mgRhB+200mg vit c/kg BW	2.28±0.12^{e}	12.15±0.94^{b}	20.89±1.23^{b}
LSD	**0.34**	**1.02**	**2.11**

Means in the same row with different superscript letters are significantly different($p \leq 0.05$).

(RhB) Rhodamine B, (TH) Testosterone, (LH) luteinizing hormone and (FSH) Follicle-Stimulating Hormone.

The effect of Rhodamine B Dye with or without V.C on Testicle CAT, SOD and MDA of male rats

The mean values of oxidative enzymes like catalase (CAT), superoxide dismutase (SOD) and malondialdehyde (MDA) as affecting

by different levels of RHB dye with and without vitamin C were delineated in table (2).

Upon the control group, it was observed that it significantly had the highest mean values of catalase and superoxidase, which were 19.42ng-mg and 115.05U-mg, respectively, while significantly had the lowest value of malondialdehyde ($p \leq 0.05$), as its value was 0.93nmol-mg. There were statistically significant gaps across all outcome measures discernible between the groups received the color only and groups supplemented the color and vitamin C at the same doses of color. No significant changes between the groups received 10 mg RhB and 20 mg RhB+200mg Vit. C/kg BW for catalase and superoxidase.

From these results, it could be observed that rhodamine b caused an increase in MDA which is one of the end products of the oxidation of polyunsaturated fatty acids in cells, the highest value was for the group that received 30 mg rhodamine/200 g BW, as it was 4.23nmol-mg. An increase in the color dose increases free radicals generate overproduction of MDA which is known as an indicator of oxidative stress and the antioxidant status. On the contrary, the color decreased both CAT and SOD which enzymes that protect cells from radical attack, the lowest value was for the group that received 30 mg rhodamine/200 g BW, as it was 8.01ng-mg and 75.29U-mg, respectively. Recent findings indicate that significant exposure to artificial colorants, such as RHB, may possess neuro-invasive properties that can trigger neurodegeneration or disorders associated with neuropsychiatric conditions, as evidenced by elevated biomarkers of oxidative and neuroinflammatory stress. This information has prompted the development of experimental models utilizing animals exposed to colorants to replicate various characteristics of distinct neurodegenerative diseases. Consequently, excessive consumption of food colorants or dyes could facilitate the production and release of inflammatory biomarkers **(Doguc et al., 2019 and Albasher et al., 2020).**

Dewi and Santi (2020) state that rhodamine B is a component of xenobiotics that the body metabolizes through cytochrome P450 to form free radicals, which in turn impact the activity of superoxidase dismutase (SOD) and cause oxidative stress, damage, and an increase in cell death.

Adding vitamin C improved these enzymes and decreased the values of MDA, the value was lowest for the group that received 10 mg Rhodamine B/200 g BWwith 200 mg Vitamin C/kg BW, where it was 2.07nmol-mg, Vit C is an essential biomolecule that plays a vital role in safeguarding cellular components against oxidative damage induced by reactive oxygen species (ROS). This vit exhibits significant antioxidant properties, as it donates a hydrogen atom, resulting in the formation of a relatively stable ascorbyl free radical. Vitamin C has the potential to

mitigate oxidative stress and, as a result, reduce the likelihood of chronic diseases, which may be linked to the consumption of colorful products.

According to M**ahmoudabadi and Rahbar (2014), & Nasef and El-Sheikh (2023)** reported that antioxidants such vitamins C, E, lipoic acid, and glutathione can lower superoxide anions via modifying antioxidant enzyme activity (SOD and GPx).

Table (2). The effect of Rhodamine B Dye with and without Vitamin C on Testicle CAT, SOD and MDA of male rats

Parameters / Groups	CAT ng-mg	SOD U-mg	MDA nmol-mg
Negative control	19.42±0.17^{a}	115.05±0.05^{a}	0.93±0.59^{f}
10 mg RhB	16.04±0.21^{c}	100.24±0.55^{c}	1.23±1.45^{e}
20 mg RhB	13.32±0.07^{d}	91.67±0.94^{d}	2.11±2.02^{d}
30mg RhB	8.01±0.23^{f}	75.29±0.87^{f}	4.23±0.04^{a}
10mgRhB+200mg vit c/kg BW	17.11±0.11^{b}	109.31±1.01^{b}	2.07±1.34^{d}
20mgRhB+200mg vit c/kg BW	15.73±0.06^{c}	98.44±0.66^{c}	3.47±0.78^{c}
30mgRhB+200mg vit c/kg BW	11.18±0.12^{e}	84.05±0.94^{e}	4.09±1.23^{b}
LSD	**1.02**	**2.31**	**0.09**

Means in the same row with different superscript letters are significantly different($p \leq 0.05$).

(RhB) Rhodamine B, (CAT)Catalase, (SOD)Superoxide dismutase and (MAD)malondialdehyde

The effect of Rhodamine B Dye with and without Vitamin C on liver liver enzymes of male rats

Data in table (3) show the effect of Rhodamine B Dye with or without Vitamin C on liver enzymes like ALT and AST of male rats. It could be noticed that control group significantly had the lowest mean values of both liver enzymes while, group fed basal diet and 30 mg RB significantly recorded the highest mean values of these enzymes, the increasing percentage was about 63% for ALT and 76% for AST as compared to control group. From the same table, it was reported that the adding of V.C led to decrease the adverse effect of RB on liver enzymes when compared their mean values at the same doses with significant differences. There are no significant differences between group fed basal diet with 30 mg RhB+200mg vit c/kg BW and the group received 20 mg RhB, also, no significant was detected between groups received 20 mg RhB+200mg vit c/kg BW and the group received 10 mg RhB. the obtained results from this table revealed that the RHB dye had negative effect on liver enzymes and this effect was increased with the increasing of dye doses, whereas rats that received Vit.C with the dye led to decrease the negative effect to its effect at the lower dose.

According to **Huang *et al.*, (2006)**, AST and ALT are released into the bloodstream when a body part or organ, such the liver or heart, is ill

or injured, which raises the enzyme's levels. As a result, the degree of tissue damage is closely correlated with the levels of AST and ALT in the blood. However, the ratio of AST to ALT, or AST/ALT, can occasionally be used to assess if damage has occurred to the liver or another organ Therefore, the elevation of ALT and AST, as shown in Table (3) indicates the effect of rhodamine B dye on liver enzymes and the occurrence of a defect in liver function.

The obtained results were matched with Many previous reports which showed that Rhodamine dye, has been shown to obstruct the import of the precursor for pyridine dinucleotide transhydrogenase into the mitochondria of rat hepatic cells and may interfere with the transhydrogenase precursor's mitochondrial import by disrupting essential components of the mitochondrial membrane that facilitate effective import. Upon the introduction of the dye to a mitochondrial suspension, respiration was initially stimulated, followed by inhibition. The failure of FCCP, a well-known uncoupler, to enhance respiration during the inhibitory phase indicates that rhodamine primarily hampers respiration through the electron transport chain rather than via the ATPase (**Parasrampuria and Mehvar, 2008).**

Vit C serves as a vital antioxidant, proficient in neutralizing free radicals. Furthermore, it fulfills numerous critical functions in enzymatic processes as a reducing agent. Research indicates that vitamin C may play a role in the regulation of hepatic and systemic lipid balance, thereby supporting the notion that vitamin C can offer protective benefits against various conditions (**Musso et al., 2003 and Luo et al., 2022).** The administration of vit. C has been shown to significantly lower other liver enzymes, including serum AST and ALT levels, while also mitigating oxidative stress associated with hepatotoxicity, indicating that this antioxidant vitamin offers protective benefits against liver injury. The incorporation of ascorbic acid supplements yields positive effects on biochemical markers, as oxidative stress can adversely impact host inflammatory processes and exacerbate various diseases, particularly those triggered by viral proteins. By effectively elevating antioxidant levels within the body, it is possible to reduce the likelihood of hepatocellular toxicity, thereby minimizing liver damage and other inflammatory responses. The role of oxidative stress in hepatitis is well established, as various oxidized proteins inflict damage on hepatic cells, resulting in necrosis and inflammation. Numerous biochemical investigations have demonstrated that ascorbic acid (vitamin C) acts as an effective antioxidant, exerting its influence by targeting free reactive oxygen species (ROS). Furthermore, additional research has confirmed the protective effects against hepatic oxidative damage attributable to the

antioxidant properties of ascorbic acid and other vitamins **(Mossa et al., 2011 and Iffat, 2020).**

Table (3). The influence of Rhodamine B dye with and without Vitamin C on liver enzymes of male rats

Treatment / Groups	ALT U/L	AST U/L
Negative control	31.34 ±1.93 ^{e}	32.66 ±0.89^{e}
10 mg RhB	39.51 ±0.45 ^{c}	40.05 ±0.83^{c}
20 mg RhB	43.79 ±1.03 ^{b}	47.11 ±1.14 ^{b}
30mg RhB	51.07 ±1.09 ^{a}	57.48 ±1.02 ^{a}
10 mg RhB+200mg vit c/kg BW	34.19 ±0.86 ^{d}	35.22 ±1.45 ^{d}
20 mg RhB+200mg vit c/kg BW	39.47 ±0.28 ^{c}	41.01 ±0.37 ^{c}
30 mg RhB+200mg vit c/kg BW	45.02 ±1.45^{b}	50.17 ±1.97^{b}
LSD	**2.05**	**3.11**

Means in the same row with different superscript letters are significantly different($p \leq 0.05$)

(RhB)Rhodamine B, (ALT) Alanine aminotransferase and (AST) Aspartate aminotransferase.

The effect of Rhodamine B dye with and without V.C on lipid profile of male rats

The effect of RhB administration with or without vitamin C on total cholesterol, triglycerides and lipid fractions of rats is presented in table (4). The results indicated that the control group significantly had lower lipid profile than the other groups except HDL which was higher than the others. The mean values were 88.93, 93.05, 21.09 and 18.61for TC, TG, LDL-c and VLDL-c, respectively while the mean value of HDL-c was 49.23 mg/dl. While the group treated with the high dose of artificial color, 30mg RhB, significantly caused the highest mean value of the previous parameters except mean value of HDL-c which significantly was the lowest. The mean values were 141.56, 186.31, 73.17 and 37.26 mg/dl for TC, TG, LDL-c and VLDL-c, respectively and 31.13 mg/dl for HDL-c. From the same table, it was observed that adding the vitamin C led to decrease the mean values of previous parameters as compared their results without the vitamin and the adding vitamin C caused mean values nearly to mean values of the low dose without the vitamin or decrease the adverse effect of the color dose. For instance, the mean values of 10 mg RhB group and 20 mg RhB+200mg vit c/kg BW group were statically non-significant, which being123.90 & 127.86 for TC, 42.15 & 42.01 mg/dl for HDL-c, 56.74 & 57.48 mg/dl for LDL-c and finally 25.01& 28.37mg/dl respectively for VLDL-c.

These results agree with those reported by **Baldev *et al.*, (2013)** who noted considerable rises in serum total lipids, cholesterol, and

triglycerides in rats given various concentrations of certain food colorants like RhB. Our findings align with those reported by Wopara et al. (2021), who found a notable increase in serum triglycerides in rats administered synthetic colorants such as RhB, tartrazine, and chocolate color containing tartrazine and carmoisine.

Vitamin C, a known antioxidant agent, exerts its protective effect by scavenging free radicals. In the study, the oral administration of vitamin C demonstrated an improvement in the condition of hyperlipidemia. Our findings are consistent with the study by **Rahimia et al. (2005)**, which suggested that the application of antioxidants mitigates oxidative stress. Vitamin C not only reduced lipid peroxidation but also enhanced the activity of antioxidant enzymes, corroborating the results of **Kedziora-Kornatowskaa *et al.* (2003)** conducted in rats. The study revealed that vitamin C significantly lowered elevated cholesterol, triglycerides, and low-density lipoprotein (LDL-c) levels. In this research, the efficacy of vitamin C against lipid peroxidation was evident through its ability to decrease the vulnerability of erythrocytes to hydrogen peroxide-induced lipid peroxidation, acting as a potent lipophilic agent that constitutes a crucial scavenger component of the cell membrane. This may safeguard membrane integrity by diminishing the formation of lipid peroxides. Furthermore, vitamin C may inhibit LDL-c oxidation while maintaining the protective antioxidant properties of HDL-c **(Badr *et al.*, 2011).**

Table (4): The effect of Rhodamine B Dye with and without Vit. C on lipid profile of male rats

Groups	T.C mg/dl	T.G mg/dl	HDL-c mg/dl	LDL-c mg/dl	VLDL-c mg/dl
Negative control	88.93±1.66^{e}	93.05 ±0.15^{g}	49.23 ±0.12^{a}	21.09 ±0.33^{e}	18.61±1.04^{e}
10 mg RhB	123.90 ±2.75^{c}	125.03 ±0.83^{e}	42.15 ±1.25^{c}	56.74±4.14^{c}	25.01±1.11^{c}
20 mg RhB	133.39±1.01^{b}	152.14 ±3.63^{c}	37.34 ±1.38^{d}	65.62 ±0.19^{b}	30.43±0.91^{b}
30mg RhB	141.56 ±1.31^{a}	186.31 ±1.65^{a}	31.13 ±2.27^{e}	73.17 ±1.34^{a}	37.26±0.26^{a}
10mg RhB+200mg vit c/kg BW	112.36 ±3.24^{d}	111.97 ±1.55^{f}	45.04 ±0.18^{b}	44.93 ±0.66^{d}	22.39±0.88^{d}
20mg RhB+200mg vit c/kg BW	127.86 ±1.97^{c}	141.85 ±1.33^{d}	42.01 ±0.63^{c}	57.48 ±3.04^{c}	28.37±1.07^{c}
30mg RhB+200mg vit c/kg BW	134.64 ±2.56^{b}	171.07 ±0.15^{b}	38.94 ±0.92^{d}	61.59 ±0.33^{b}	34.21±1.04^{b}
LSD	**3.99**	**6.23**	**3.01**	**5.11**	**3.78**

**Means in the same row with different superscript letters are significantly different($p \leq 0.05$).*

**(RhB) Rhodamine B,(T.C) Total Cholesterol,(T.G)Triglycerides,(HDL)High-density lipoprotein,(LDL) Low-density lipoprotein and (VLDL)very Low-density lipoprotein.*

The effect of Rhodamine B dye with and without Vit.C on hematological parameters of male rats

Data presented in table (5) revealed that the effect of RHB color with or without vitamin C on the hematological parameters of normal rats. it was found that all mean values of hematological parameters were significantly lower than the control group and the reduction was significantly increase with increasing the dose of the color. In contrast to that the white blood cell was significantly increased with increasing the color dose. The most adverse effect was recorded in the group treated with 30 mg, the color followed 20 mg and 10 mg which had the lowest negative effect. Adding vitamin C to the groups treated with the different levels of color led to decrease the side effect of the color on these parameters when compared with the same levels of the color with statically significant differences.

For RBCs, the mean value of the highest dose group without Vitamin C was 3.81(10e/mm3) while the mean value of the same dose with vitamin C was 4.14(10e/mm3) by improvement percentage 6.88%. In case of the other parameters were 4.25, 13.27, 11.38, 20.88, 7.49 and 8.24% for HGB, HCT, MCV, MCH, MCHC and PLT respectively whereas the reduction percentage of WBC was 16.17%.

The administration of the color for 1 month, induced a decrease in the rats of RBCs, Hb% and Hct% and increase the level of WBCs. It is known that to increase the free radical which transforms the ferrous ion of hemoglobin into a ferric ion both in vivo and in vitro. This phenomenon elucidates the decline in hemoglobin concentrations. In other words, introducing the dye triggers a condition of inadequate oxygen in the tissues that generate blood, ultimately leading to a slowdown in red blood cell production and thereby lowering hemoglobin levels in the bloodstream. The decline in hemoglobin as a result of pigment exposure has been documented in various animal models, including rats, mice, dogs, swine, and sheep. Additionally, RHB has been observed to cause a reduction in hemoglobin levels in human subjects. The overall count of erythrocytes and hemoglobin levels had diminished. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC), along with erythrocyte sedimentation rates (E.S.R.), had also shown a decline. These observations indicated the presence of normochromic macrocytic anemia. The differential leukocyte count revealed a significant rise in lymphocytes and monocytes, accompanied by a drop in neutrophils and eosinophils. A decrease in total white blood

cell counts was noted following caramel treatment in rats (**Doguc *et al.*, 2019; Albasher *et al.*, 2020; and Wopara *et al.*, 2021).**

Vitamin C has demonstrated significant antioxidant capabilities that shield against damage caused by free radicals, thus maintaining the body's optimal functioning. Therefore, it is highly advisable to incorporate a moderate daily intake of vitamin C in the diets of both children and adults, as it offers protection against pro-oxidants and other harmful impacts associated with food additives and various food toxins. Additionally, vitamin C can safeguard vascular endothelial cells from oxidative damage. Vitamins C contain compounds, making it a potent antioxidant, protecting cell membranes and DNA from damage and disease. Vitamin C supplements may enhance the body's ability to absorb dietary iron. This vitamin plays a crucial role in transforming less absorbable iron, particularly from plant sources, into a more readily absorbable form (**Rahimia *et al.*, 2005 and Chunli *et al.*, 2024).**

Table (5A). The effect of Rhodamine B dye with and without Vit.C on hematological parameters of male rat

Groups	RBC(10e/mm3)	HGB(g /dl)	HCT (%)	MCV(um3)
Negative control	5.04±0.07 [a]	12.25±0.029 [a]	40.27 ±0.56[a]	98.01±1.02 a
10 mg RhB	4.67±0.08 [c]	11.12±0.032 [c]	35.72 ±1.39[c]	88.86±2.15 [c]
20 mg RhB	4.23±0.06 [e]	10.63±0.007 [e]	30.06 ±0.71[e]	78.03±1.22 [e]
30mg RhB	3.81±0.11 [f]	10.12±0.001[f]	24.71 ±1.21[f]	70.04±1.74 [f]
10 mg RhB+200mg vit c/kg BW	4.88±0.03 [b]	11.24±0.05 [b]	38.14 ±0.79[b]	93.90±0.47[b]
20 mg RhB+200mg vit c/kg BW	4.42±0.04 [d]	10.93±0.04 [d]	32.81 ±1.04[d]	84.01±1.01[d]
30 mg RhB+200mg vit c/kg BW	4.14±0.07 [e]	10.55±0.07 [e]	27.99 ±0.79[e]	78.01±2.92[e]
LSD	0.11	0.09	2.11	4.03

Means in the same row with different superscript letters are significantly different($p \leq 0.05$).

(RhB)Rhodamine B,(RBC)Red blood cells, (HGB)Hemoglobin, (HCT)Hematocrit and (MCV) corpuscular volume.

Table (5B). The effect of Rhodamine B dye with and without Vit.C on hematological parameters of male rat

Groups	MCH (pg)	MCHc (g /dl)	PLT(10e/mm3)	WBC(10e /mm3)
Negative control	34.95±0.31 [a]	35.21±1.46[a]	195.01 ±0.61[a]	4.04±0.17 [f]
10 mg RhB	28.33±0.16[c]	30.56±1.62 [c]	184.6 5±2.41[c]	5.11±0.07[c]
20 mg RhB	22.45±0.004 [e]	27.66±0.55 [e]	172.31 ±1.23[e]	6.43±0.01[b]
30mg RhB	18.53±0.83[f]	25. 23±0.33 [f]	157.76 ±0.64[f]	7.79±0.13 [a]
10 mg RhB+200mg vit c/kg BW	31.02±1.28[b]	32.11±1.52 [b]	189.71±1.04[b]	4.55±0.19[e]
20 mg RhB+200mg vit c/kg BW	25.38±1.06[d]	29.11±0.08[d]	178.64 ±2.01[d]	4.83±0.08[d]

30 mg RhB+200mg vit c/kg BW	22.40±1.21 [e]	27.12±1.28[e]	170.74±2.81[e]	6.53±0.11[b]
LSD	2.55	1.43	4.21	0.25

Means in the same row with different superscript letters are significantly different ($p \leq 0.05$).

(MCH) corpuscular Hemoglobin, (MCHc) hemoglobin concentration, (PLT) Platelets and (WBC)White blood cells.

The effect of Rhodamine B dye with and without Vit.C on FI. BW and FER of male rats

Table (6) presented the mean values of body weight, feed intake and feed efficiency ratio as affected by treated with RHB color with or without vitamin C. Data showed high weight gain, feed intake and feed efficiency ratio of Control group which being , 38.11%, 14.03g/d and 0.097%g respectively; while the gain of body weight in rats treated with different doses of RhB was decreased by the percentage 19.16, 32.20 and 53.52% respectively for groups received 10,20 and 30 mg as compared to the control group and in case the feed intake was 14.40, 30.75 and 42.20% respectively. The decreasing of weight and feed intake led to a decrease in the feed efficiency ratio. supplemented the color with vitamin C significantly led to high the previous parameters as compared to the same doses groups without vitamin C ($p \leq 0.05$). The improvement percentages as compared to the previous groups were 8.90, 4.47 and 13.81% in case the feed intake, in case of body weight, Were 11.27, 8.13 and 19.65 %. No significant ($P<0.05$) changes between groups 2 and 6 regarding to feed intake and body weight while, it was found between groups 2 and 5 regarding to FER.

The current findings regarding weight loss following the administration of food colorants may be attributed to a decrease in food intake. Conversely, the observed decline in average body weight could result from elevated colorant dosage, which may enhance catabolic activity within the organism. The reduction in weight gain was noted as a consequence of color treatments. Numerous researchers have documented a decrease in body weight associated with the supplementation of colorants **(Baldev *et al.*, 2013 and Wopara *et al.*, 2021).** Antioxidants as vitamin C are molecules that boost the immune system, fight inflammation and protect cells from harmful molecules called free radicals. It can help gain weight. It's a necessary vitamin to slow metabolism and help keep overeating. Appetite increases in response to body weight owing to differences in hormone levels that regulate energy balance, such as insulin, leptin, and cortisol.

Vitamin C is involved in the production of these and other peptides and neurotransmitters that regulate food intake. Vitamin C is needed for the

oxidation of energy substrates and must be obtained by the diet to increase energy intake **(Geneviève *et al*., 2008).**

Table (6). The effect of Rhodamine B dye with and without Vit.C on FI. BWG and FER of male rat

Treatments / Groups	Feed intake g/day	Body weight %	FER %
Negative control	14.03 ±0.38 [a]	38.11±0.92 [a]	0.097±0.001[a]
10 mg RhB	12.01±0.12 [c]	30.61±0.83[c]	0.091±0.002 [b]
20 mg RhB	10.73±0.05 [d]	25.84±0.25[d]	0.086±0.001[d]
30mg RhB	8.11±0.01 [f]	17.71±0.63 [f]	0.078±0.001 [f]
10 mg RhB+200mg vit. c/kg BW	13.08±0.28[b]	34.06±0.86 [b]	0.093±0.002[b]
20 mg RhB+200mg vit. c/kg BW	11.21±0.11[c]	27.94±0.62 [c]	0.089±0.001 [c]
30 mg RhB+200mg vit. c/kg BW	9.23 ±0.38 [e]	21.19±0.42 [e]	0.082±0.002[e]
LSD	**1.09**	**3.39**	**0.002**

Means in the same row with different superscript letters are significantly different($p \leq 0.05$).

(RhB) Rhodamine B, (BWG) Body weight gain, (FI) Feed intake, (FER) feed efficiency ratio.

Histopathological examination of testes:

The effect of using Rhodamine B stain on the histological examination of Testicles in male rats was demonstrated.

Microscopically, Testicles of rats from group 1 showed the normal histological structure of seminiferous tubule with normal spermatogonia cells and complete spermatogenesis (photos 1, 2 & 3). In contrariwise, testes of rats from group 2 exhibited degeneration of spermatogonia cells lining some seminiferous tubules (photos. 4 & 5) and interstitial edema (photo. 5). Furthermore, Testicles of rats from group 3 revealed degeneration of spermatogonia cells lining some seminiferous tubules (photos. 6 & 7) and congestion of interstitial blood vessels (photo. 7). Moreover, examined sections from group 4 described degeneration of spermatogonia cells lining some seminiferous tubules (photos. 8 & 9). Otherwise, Testicles of rats from group 5 exhibited no histopathological alterations (photos.10, 11& 12). On the other hand, some examined sections from group 6 revealed degeneration of spermatogonia cells lining some seminiferous tubules (photos. 13 & 14) with appearance of spermatid giant cells (photo. 14), whereas other sections from this group exhibited histologically normal seminiferous tubules (photos. 15 & 16). Furthermore, Testicles of rats from group 7 showed no histopathological lesions with histologically normal seminiferous tubules (photos. 17, 18, 19 & 20).

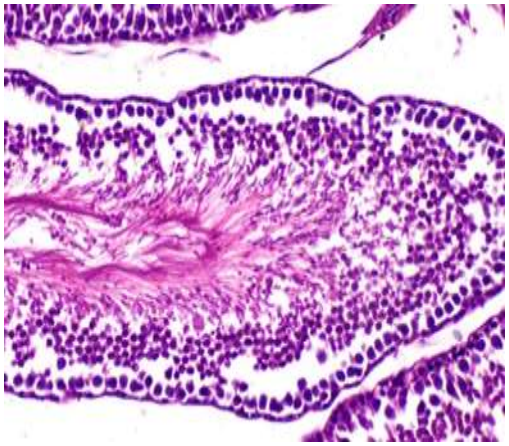


Photo. (1). Photomicrograph of testis of rat from group 1 showing the normal histological of seminiferous tubule with normal structure of spermatogoneal cells and complete spermatogenesis.

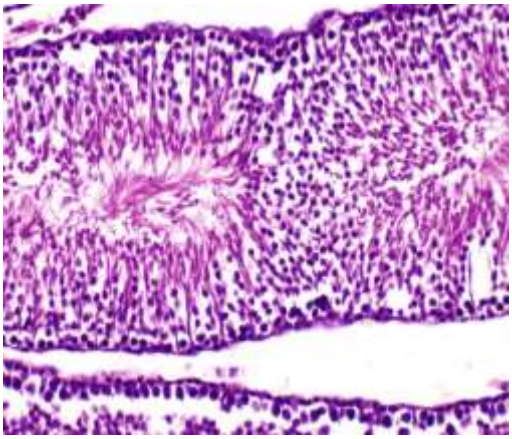


Photo. (2). Photomicrograph of testis of rat from group 1 showing the normal histological structure seminiferous tubule with normal spermatogoneal cells and complete spermatogenesis.

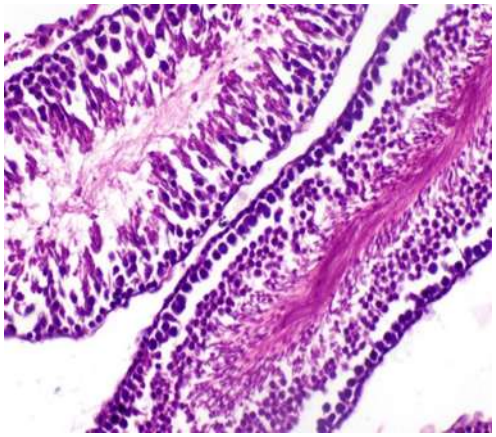


photo. (3). Photomicrograph of testis of rat from group 1 showing the normal histological structure of seminiferous tubule with normal spermatogoneal cells and complete spermatogenesis.

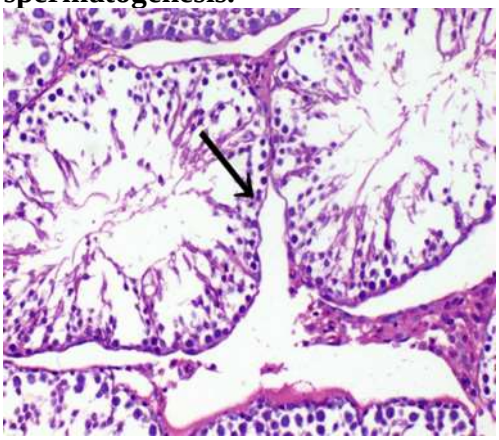


photo. (4). Photomicrograph of testis of rat from group 2 showing degeneration of spermatogoneal cells lining some seminiferous tubules (black arrow).

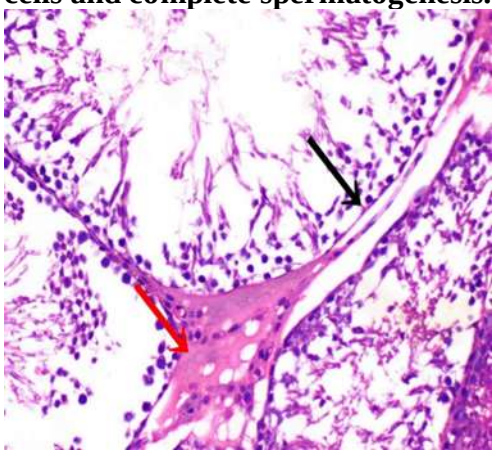
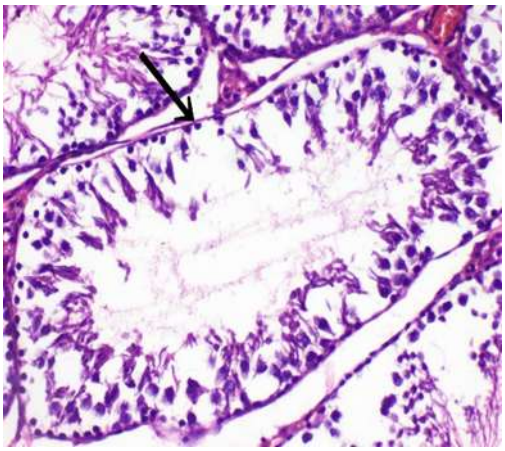


photo. (5). Photomicrograph of testis of rat from group2 showing degeneration of spermatogoneal cells lining some



group2 photo. (6). Photomicrograph of testis of rat from group3 showing degeneration of spermatogoneal cells

seminiferous tubules (black arrow) and interstitial edema (red arrow).

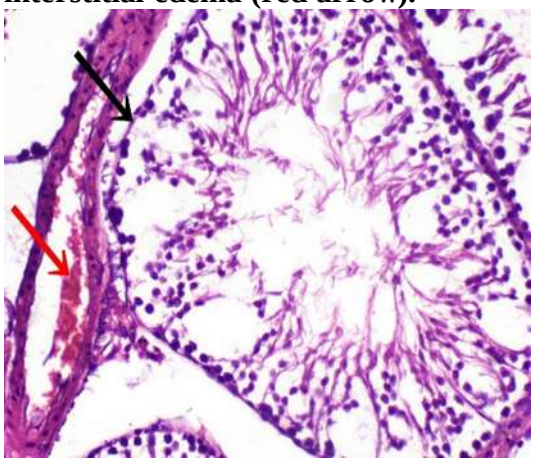


photo. (7). Photomicrograph of testis of rat from group3 showing degeneration of spermatogoneal cells lining some seminiferous tubules (black arrow) and congestion of interstitial blood vessel (red arrow).

lining some seminiferous tubules (black arrow).

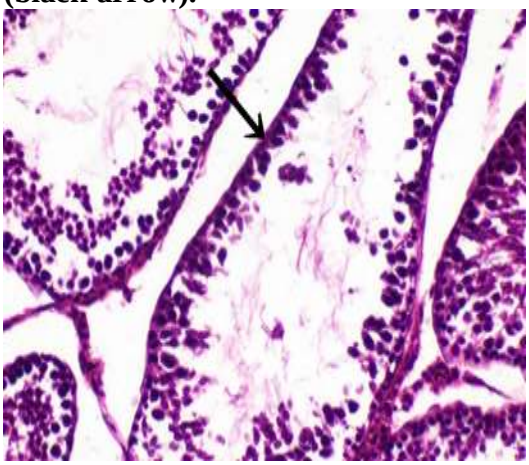


photo. (8). Photomicrograph of testis of rat from group4 showing degeneration of spermatogoneal cells lining some seminiferous tubules (black arrow).

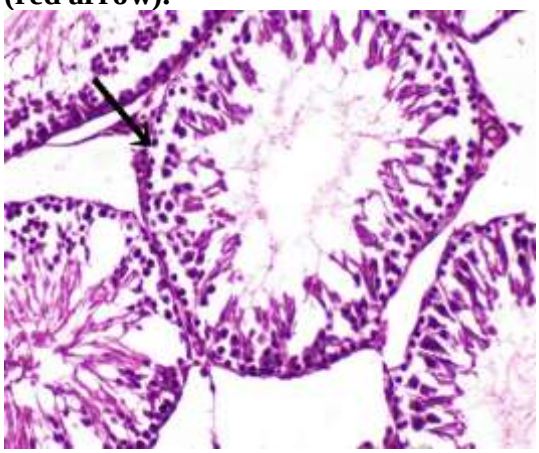


photo. (9). Photomicrograph of testis of rat from group4 showing degeneration of spermatogoneal cells lining some seminiferous tubules (black arrow).

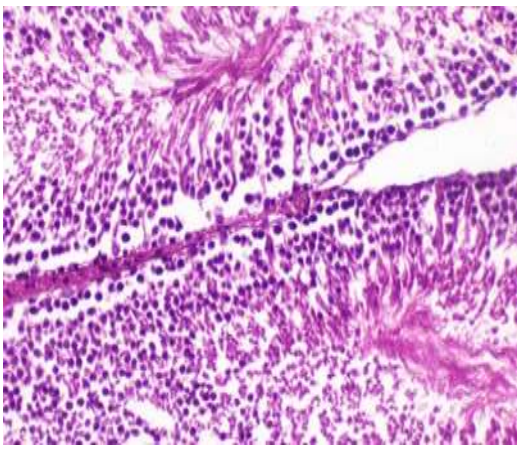


photo. (10). Photomicrograph of testis of rat from group 5 showing no histopathological alterations.

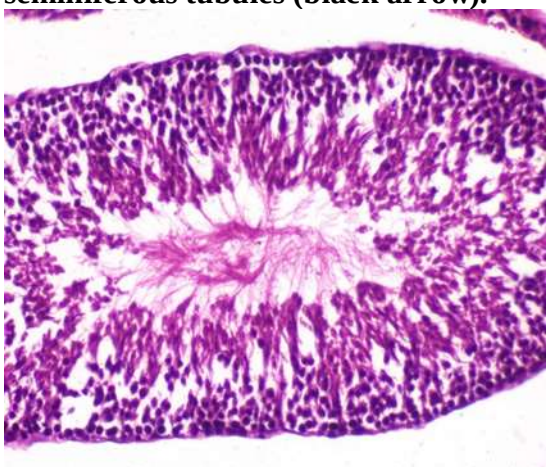


photo. (11). Photomicrograph of testis

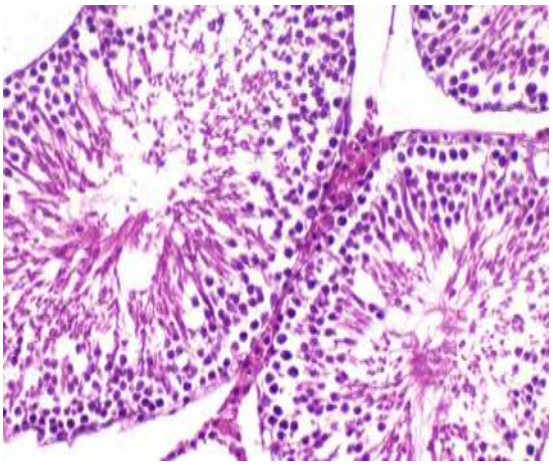


photo. (12). Photomicrograph of testis

of rat from group 5 showing no histopathological alterations (H & E X 400).

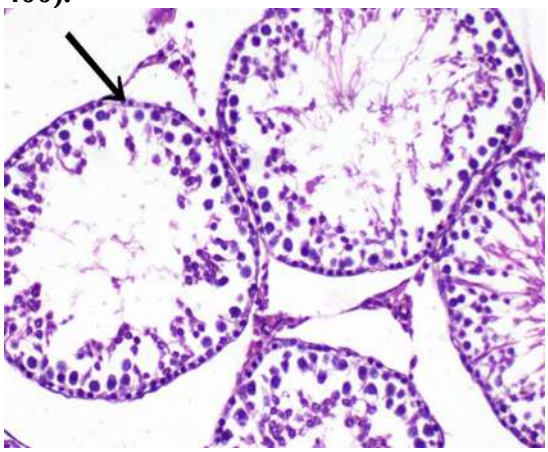


photo. (13). Photomicrograph of testis of rat from group 6 showing degeneration of spermatogoneal cells lining some seminiferous tubules (black arrow) (H & E X 400).

of rat from group 5 showing no histopathological alterations (H & E X 400).

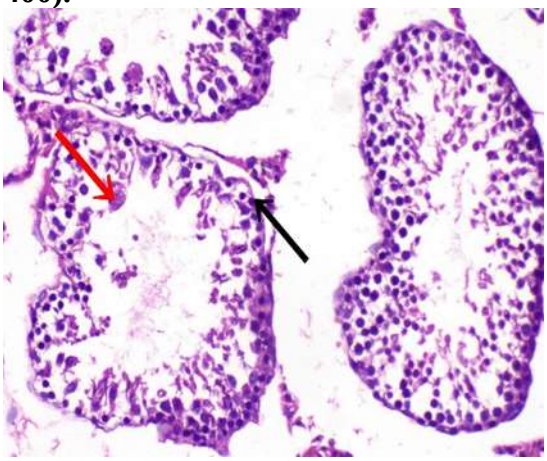


photo. (14). Photomicrograph of testis of rat from group 6 showing degeneration of spermatogoneal cells lining some seminiferous tubules (black arrow) with appearance of spermatid giant cells (red arrow).

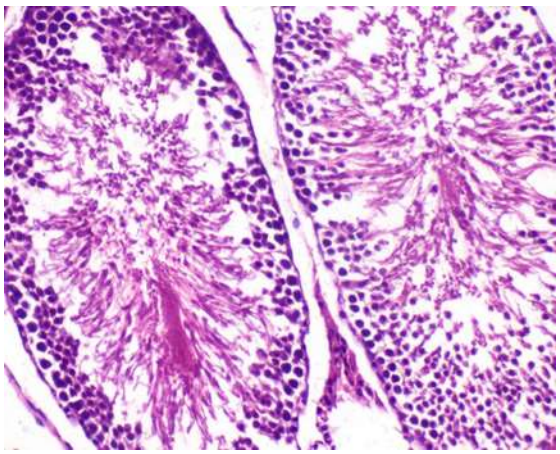


photo. (15). Photomicrograph of testis of rat from group 6 showing histologically normal seminiferous tubules.

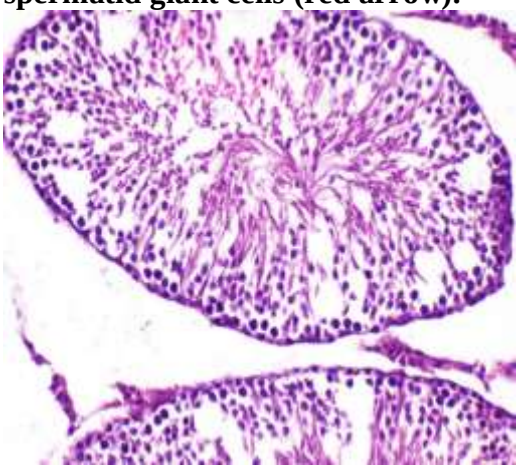


photo. (16). Photomicrograph of testis of rat from group 6 showing histologically normal seminiferous tubules.

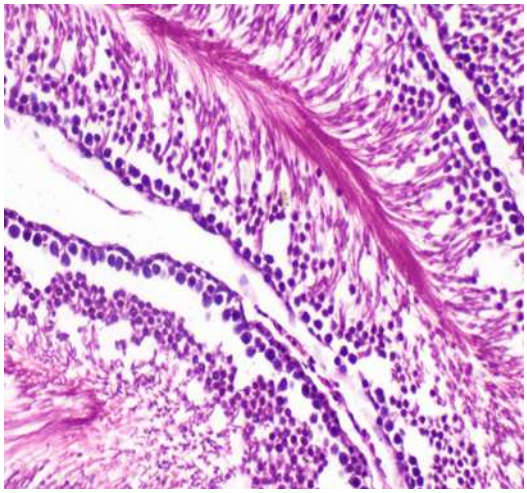


photo. (17). Photomicrograph of testis of rat from group 7 showing histologically normal seminiferous tubules.

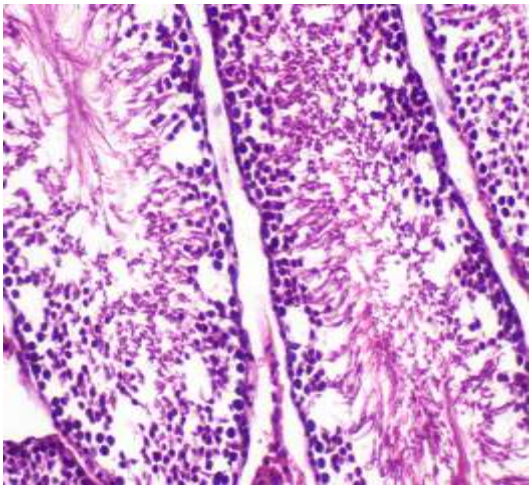


photo. (18). Photomicrograph of testis of rat from group 7 showing histologically normal seminiferous tubules.

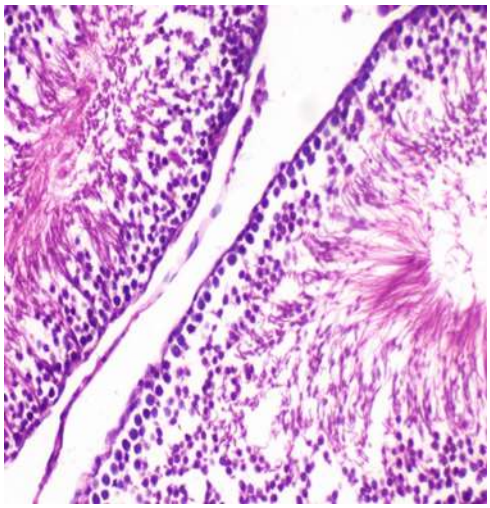


photo. (19). Photomicrograph of testis of rat from group 7 showing histologically normal seminiferous tubules.

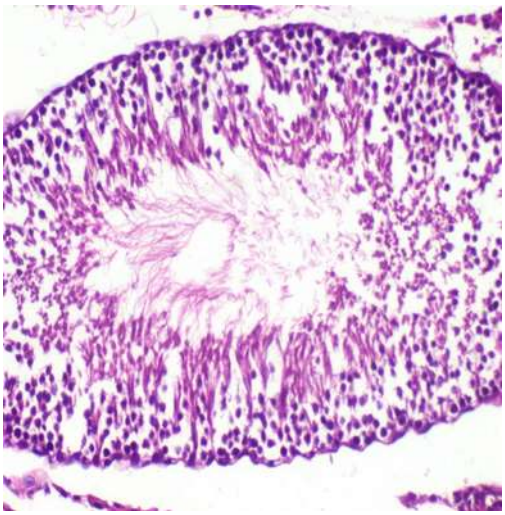


photo. (20). Photomicrograph of testis of rat from group 7 showing histologically normal seminiferous tubules.

Conclusions

In conclusion, the ability of Rhodamine B to directly affect testicular tissues and their biomarkers including organ weight, hormone levels as well as other organs in rats was verified with increasing dose. Rhodamine B administration leads to decreased reproductive capacity.

The obtained results indicate the potential effectiveness of kiwi fruits as a source of vitamin C as a protective agent against the harmful effects of Rhodamine B on testes and other organs. Antioxidants reduce testicular injury by reducing oxidative stress, apoptosis and inflammation.

References

A.R.E. National Nutrition Institute. (2006): food composition tables for Egypt, second edition.

Adriani, A. P. (2019): Untuk Masyarakat Tentang Bahaya Pewarna Melalui Publikasi Hasil Analisis Kualitatif Pewarna Sintesis Dalam Saus. Jurnal Serambi Ilmu; 20(2).

Aebi, H. (1984): Catalase in vitro. InMethods in enzymology Jan 1 (Vol. 105, pp. 121-126): Academic press.

Agarwal, A.; Mulgund, A.; Hamada, A. and Chyatte, M.R. (2015): A unique view on male infertility around the globe. Reprod Biol Endocrinol. Apr 26;13:37.

Ahn, Y.; Lee, Y.; Park, H. and Song, K. (2021): Gender and age group differences in nutrition intake and dietary quality of Korean adults eating alone: based on Korean national health and nutrition examination survey data, 2013–2016. Nutr Res Pract. 15(1):66–79.

Ainslie L.K. Roberts , Janice M. Fletcher , Lynette Moore and Sharon Byers(November 2010):" Trans-generational exposure to low levels of rhodamine B does not adversely affect litter size or liver function in murine mucopolysaccharidosis type IIIA"Received 27 April 2010, Revised 11 June 2010, Accepted 11 June 2010, Available online 22 June 2010, Pages 208-213.

Albasher,G.; Maashi,N.; Alfarraj,S.; Almeer, R.; Albrahim,T.; Alotibi,F.; Bin-Jumah, M. and Mahmoud, A.M. (2020) : Perinatal exposure to tartrazine triggers oxidative stress and neurobehavioral alterations in mice offspring Antioxidants, 9 (1): 53.

Allain, C.C.; Poon, L.S.; Chan, C.S.; Richmond, W.F. and Fu, P.C. (1974): Enzymatic determination of total serum cholesterol. Clinical chemistry. 20(4):470- 5.

Aly, N.; El-Gendy, K.; Mahmoud, F. and ElSebae, A.K. (2010): Protective effect of vitamin C against chlorpyrifos oxidative stress in male mice. Pesticide Biochem. Physiol.; 97: 712.

Badr, G.; Bashandy, S.; Ebaid, H.; Mohany, M., and Sayed, D. (2011): Vitamin C supplementation reconstitutes polyfunctional T cells in streptozotocin-induced diabetic rats. Eur. Nutr, 25.

Baldev, E.; Mubarak,A.D.; Llavarasi, A.; Pandiaraj, D.; Ishack, K.A. Thajuddin N.(2013): Degradation of synthetic dye, rhodamin B to environmentally non-toxic products using microalgae. Colloids and Surfaces B: Biointerfase 105: 207-214.

Bancroft, J.D. and Gamble, M. (2008): Theory and practice of histological techniques. Elsevier health sciences.

Boein,. H.; Bechthold, A.; Bub, A.; Ellinger, S.; Haller, D.; Kroke, A.; Leschik-Bonnet, E.; Müller, M.J.; Oberritter, H.; Schulze, M.;Stehle, P. and Watzl, B.(2012): Critical review: vegetables and fruit in the prevention of chronic diseases. Eur J Nutr. 51:637–663.

Boland, M. (2013): Nutritional Benefits of Kiwifruit. Kiwifruit Proteins and Enzymes; pp. 59–80.

Carr, A.C. and Rowe, S. (2020): Factors affecting vitamin C status and prevalence of deficiency: a global health perspective. Nutrients. 12(7):1963.

Chapman, D.G.; Castillo, R. and Campbell JA. (1959 May): Evaluation of protein in foods: 1. A method for the determination of protein efficiency ratios. Canadian Journal of Biochemistry and Physiology. 1;37(5):679-86.

Chunli, W.; Jili, Z.; Qiaoqiao, Z. and Jing, L. (2024): Serum vitamin C levels and their correlation with chronic kidney disease in adults: a nationwide study. ;46(1):2298079.

Dacie, A. and Lewis, D. (2006): Practical Hematology. Churchill. Livingstone imprint. 10th Edition, Paperback ISBN: 9780443066603. P. 736. NewYork.

Daly, M. E. (2011): Determinants of platelet count in humans. Haematologica, 96(1), 10.

David, F.Y. and Jessy, N.M. (2016): Male infertility: Lifestyle factors and holistic, complementary, and alternative therapies. Asian Journal Androl., 18 (3): 410-418.

Dewi, R. S. and Santi, M.(2020): The effect of Rhodamine B on the cerebellum and brainstem tissue of Rattus norvegicus.1Doctoral Program of Public Health, 2Department of Epidemiology, Faculty of Public Health, Universitas Airlangga, Mulyorejo, Surabaya, Indonesia. Journal of Public Health Research; 9:1812.

Doguc, D.K.; Deniz F.; İlhan, İ. and Ergonul, F. (2019): Gultekin Prenatal exposure to artificial food colorings alters NMDA receptor subunit concentrations in rat hippocampusNutr. Neurosci. 1-11.

Fahim, M.S.; Fahim, Z. and Harman, J.M. Clevenger, T.E.; Mullins, W.; Hafez, E.S. (1982): Effect of Panax ginseng on testosterone level and prostate in male rats. Arch Androl, 8 (4): 261-263

Fassati, P. and Prencipe, L. (1982): Triglyceride enzymatic colorimetric method. J. of Clin. Chem., 28: 2077.

Geneviève, C.; Major,E.; Doucet,M.; Jacqmain,M.; St-Onge,C. B. and Angelo, T. (2008): Multivitamin and dietary supplements, body weight and appetite: results from a cross-sectional and a randomised double-blind placebo-controlled study. Published online by Cambridge University Press, British Journal of Nutrition: 99 (5) 1157 – 1167.

Goscianska, J.; Ptaszkowska, M. and Pietrzak, R. (2015): *Equilibrium* and kinetic studies of chromotrope 2R adsorption onto ordered mesoporous carbons modifed with lanthanum. Chem. Eng. J. 270, 140–149.

Hadriyati, A. (2021): Analisis Rhodamin B dalam Bolu Kukus yang Beredar di Kota Jambi dengan Metode Spektrofotometri UV-Vis. Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia; 8(1): 16.

Hafkenscheid, J.C. (1979): Determination of GOT. Clinical Chemistry, 25:155.

Hamdaoui, O., (2011): Intensifcation of the sorption of Rhodamine B from aqueous phase by loquat seeds using ultrasound. Desalination 271, 279–286.

Huang, X.; Choi, Y.; Im, H.; Yarimaga, O.; Euisik, Y, and Kim, H. (2006): (Aspartate Aminotransferase (AST/GOT) and Alanine Aminotransferase (ALT/GPT) Detection Techniques. Sensors,6: 756-782.

Iffat, N. (2020):"Effect of Ascorbic Acid Supplementation on Liver Function Tests in Hepatitis C Patients."Journal of Internal Medicine.10 (3) .

Imam, S.S. and Babamale, H.F., (2020): A short review on the removal of rhodamine B dye using agricultural waste-based adsorbents. Asian J. Chem. Sci. 7, 25–37.

Jyotshana, S.; Shubhangani, S.;Upma B.andVineet S.(2022): Toxic effects of Rhodamine B on antioxidant system and photosynthesis of Hydrilla verticillata. Journal of Hazardous Materials Letters. 3, 100069

Kaur, L.; Rutherfurd, S.M.; Moughan, P.J.; Drummond. L. and Boland, M.J. (2010): Actinidin enhances protein digestion in the small intestine as assessed using an in vitro digestion model. J Agric Food Chem. 58(8):5074–5080.

Kedziora-Kornatowska, K.; Szram, S.; Kornatowski, T.; Szadujkis-Szadurski, L.; Kedziora, J. and Bartosz, G. (2003): Effect of Vitamin E and Vitamin C Supplementation on antioxidative state and renal Glomerular basement membrane thickness in Diabetic Kidney. Experimental nephrol, 95 (4), 34-43.

Koda-Kimble, M.; Young, L.; Kradjan, W. and Gulielmo, B. (2001): Applied therapeutic: The Clinical use of drugs. 17 Lippincott, Willuans and Willkins and Wolters kluwercompay, 2:17-19.

Kusumaningrum, N. and Fitriah, F. (2020): Perancangan Pembuatan Permen Kapas Otomatis Berbasis Arduino Uno. TESLA: Jurnal Teknik Elektro; 22(1): 12.

Lee, R.D, and Nieman, D.C. (1996): Nutritional assessment 2nd. Ed. Mosby-Year Book.

Levine, H.; Jergensen, N. and MartinoAndrade, A. (2017): Temporal trends in sperm count: a systematic review and metaregression analysis. Hum Reprod Update, 23:646-659.

Lopez, M.F.(1977): HDL-cholesterol colorimetric method. J. of Clin. Chem, 23:882.

Lubsandorzhiev, B. K. (2006): On the history of photomultiplier tube invention. Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment, 567(1), 236-238.

Luo, X.; Zhang, W.; He, Z.; Yang. H.; Gao, J.; Wu, P. and Ma, Z.F. (2022). Dietary Vitamin C Intake Is Associated With Improved Liver Function and Glucose Metabolism in Chinese Adults. Front Nutr. Jan 31;8:779912.

Macan, A.; Kraljevic, T.; Raic-Mali, S. and et al .(2019): Therapeutic perspective of vitamin C and its derivatives. Antioxidants, 8: 247 .

Mahdi, C.; Pratama. C.A. and Pratiwi H. (2019):" Preventive Study Garlic Extract Water (*Allium sativum*) Toward SGPT, SGOT, and the Description of Liver Histopathology on Rat (*Rattus norvegicus*), which were exposed by Rhodamine B". *IOP Conf Ser Mater Sci Eng* ;546:062015.

Mahmoudabadi, M.M. and Rahbar, AR.(2014). Effect of EPA and vitamin C on superoxide dismutase, glutathione peroxidase, total antioxidant capacity and malondialdehyde in type 2 diabetic patients. Oman Med J.;29(1):39-45.

Mamta, P.; Kshipra, M.; Garry, D.; Satinder, K. B. and Mausam, V.(2014): Antioxidants. In book: Biotransformation of Waste Biomass into High Value Biochemicals (117-138).

Moss, D.W. (1982): Alkaline phosphatase isoenzymes. Clinical Chemistry, 28: 2007-2016.

Mossa, A.A.; Refaie, K. and Ramadan, A. (2011): Effect of Exposure to Mixture of Four Organophosphate Insecticides at No Observed Adverse Effect Level Dose on Rat Liver: The Protective Role of Vitamin C. Research Journal of Environmental Toxicology, 5, 323-335.

Musso, G.; Gambino, R.; De- Michieli, F.; Cassade,. M.; Rizzetto, M. and Durazzo, M. (2003) . Dietary habits and their relations to insulin resistance and postprandial lipemia in nonalcoholic steatohepatitis. Hepatology. 37:909–16.

Nasef, A. and El-Sheikh, N. A. (2023). Potential Effects of Horny Goat Weed (*Epimedium grandiflorum*) on The Level of Fertility in Male Rats Infected with Cadmium Chloride: Biochemical and Histopathological Study. *Alexandria Science Exchange Journal*, *44*(3), 433-443.

Paoletti, F. and Mocali, A. (1990): Determination of superoxide dismutase activity by purely chemical system based on NAD (P) H oOxidation. InMethods in enzymology . Academic press.186, : 209-220.

Parasrampuria, R. and Mehvar, R. (2008): Hepatobiliary disposition of rhodamine 123 in isolated perfused rat livers, Xenobiotica, 38(10): 1263-1273.

Predelles, P.; Grassi, J. Maclouf, J.A. (1985): Enzyme immunoassays of eicosanoids using acetylchline esterase as lable. J. Anal.Chem, 57:1170-1173.

Rahimia, R.; Nikfara, S.; Larijanib, B., and Abdollahia, M. (2005): A review on the role of antioxidants in the management of diabetes and its complications. Biomedecine & Pharmacotherapy, 59 (7), 365-373.

Rouhier, N.; Lemaire, S. and Jacquot J (2008): The role of glutathione in photosynthetic organisms: Emerging unctionsfunctions for glutaredoxins and glutathionylation. Annual Review of Plant Biology,59:143-166.

Rutstein, S.O. and Shah, I.H.(2004): Infecundity, Infertility, and Childlessness in Developing Countries (No. 9); Measure Dhs.; ORC Macro: Calverton, MD, USA; World Health Organisation: Geneva, Switzerland.

Satoh, K. (1978): Estimation of lipid peroxides by thiobarbituric acid reactive substances (TBARS): Clin Chim Acta. 90:37-43.

Schermer, A. (1967): "The Blood Morphology of Laboratory Animal". Longmans, Printed in Great Britain, Green and Co. Ltd, pp.350.

Sengupta, P.; Dutta, S. and KrajewskaKulak, E. (2017): The disappearing sperms: analysis of reports published between 1980 and 2015. American journal of men's health, 11, 1279–1304.

Siswati, P. and Slamet, J.S.(2000):" The study of hepatotoxicity from food dye on mice" (*Mus musculus*) Australian strain. Indon J Toxicol 2000;1:18.

Tjiptaningdyah, R and Sucahyo, M.B.S.(2016): Analisis Zat Pewarna Rhodamin B Pada Jajanan Yang Dipasarkan Di Lingkungan Sekolah. Agriekstensia; 16(2):

Varjani, S., Rakholiya, P., Ng, H.Y., You, S., Teixeira, J.A., (2020): Microbial degradation of dyes: an overview. Bioresour.Technol.314, 123728 .

Vuˇ curovi´ c, V.M.; Razmovski, R.N.; Milji´ c, U.D. and Puˇ skaˇ s V.S., (2014): Removal of cationic and anionic azo dyes from aqueous solutions by adsorption on maize stem tissue. J. Taiwan Inst. Chem. Eng. 45, 1700–1708.

Winters, B.R. and Walsh, T.J. (2014): The epidemiology of male infertility. Urol Clin North Am; 41:195-204.

Wopara, I.; Modo, E.U.;Mobisson, S.K.; Olusegun, G.A.; Umoren, E.B.; Orji, B.O.; Mounmbegna, P.E. and Ujunwa, S.O. (2021) Synthetic Food dyes cause testicular damage via up-regulation of pro-inflammatory cytokines and down-regulation of FSH-R and TESK-1 gene expression. JBRA Assist Reprod.;25(3):341-348.

Zegers, F. (2017): Hochschild The international glossary on infertility and fertility care, 2017 Fertil. Steril

Zhao, D.; Zhang, W.; Chen, C. and Wang, X., (2013): Adsorption of methyl orange dye onto multiwalled carbon nanotubes. Procedia Environ. Sci. 18, 890–895.